

WEST[Generate Collection](#)**Search Results - Record(s) 1 through 10 of 21 returned.**☐ 1. Document ID: US 20010021700 A1

L8: Entry 1 of 21

File: PGPB

Sep 13, 2001

PGPUB-DOCUMENT-NUMBER: 20010021700

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20010021700 A1

TITLE: 49 human secreted proteins

PUBLICATION-DATE: September 13, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Moore, Paul A.	Germantown	MD	US	
Ruben, Steven M.	Oley	MD	US	
Olsen, Henrik S.	Gaithersburg	MD	US	
Shi, Yanggu	Gaithersburg	MD	US	
Rosen, Craig A.	Laytonsville	MD	US	
Florence, Kimberly A.	Rockville	MD	US	
Soppet, Daniel R.	Centreville	VA	US	
Lafleur, David W.	Washington	DC	US	
Endress, Gregory A.	Potomac	MD	US	
Ebner, Reinhard	Gaithersburg	MD	US	
Komatsoulis, George	Silver Spring	MD	US	
Duan, Roxanne D.	Bethesda	MD	US	

US-CL-CURRENT: [514/44](#); [435/320.1](#), [435/325](#), [435/69.1](#), [435/69.7](#), [435/7.1](#), [514/12](#), [530/350](#),
[530/388.2](#), [536/23.5](#)

Full	Title	Citation	Front	Review	Classification	Date	Reference	Claims	KWIC	Draw Desc	Image
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☐ 2. Document ID: US 20010002401 A1

L8: Entry 2 of 21

File: PGPB

May 31, 2001

PGPUB-DOCUMENT-NUMBER: 20010002401
PGPUB-FILING-TYPE: new-utility
DOCUMENT-IDENTIFIER: US 20010002401 A1

TITLE: TREATING OR PREVENTING THE EARLY STAGES OF DEGENERATION OF ARTICULAR
CARTILAGE OR SUBCHONDRAL BONE IN MAMMALS USING CARPROFEN AND DERIVATIVES

PUBLICATION-DATE: May 31, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
EVANS, NIGEL A	EAST LYME	CT	US	
KILROY, CAROLYN R	OLD LYME	CT	US	
LUNDY, KRISTIN M	GROTON	CT	US	
JEAN-PIERRE, PELLETIER	ST LAMBERT		CA	

US-CL-CURRENT: 514/412

Full	Title	Citation	Front	Review	Classification	Date	Reference	Claims	KWIC	Draw Desc	Image
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☐ 3. Document ID: US 6124273 A

L8: Entry 3 of 21

File: USPT

Sep 26, 2000

US-PAT-NO: 6124273

DOCUMENT-IDENTIFIER: US 6124273 A

TITLE: Chitin hydrogels, methods of their production and use

DATE-ISSUED: September 26, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Drohan; William N.	Springfield	VA	N/A	N/A
MacPhee; Martin J.	Gaithersburg	MD	N/A	N/A
Miekka; Shirley I.	Gaithersburg	MD	N/A	N/A
Singh; Manish S.	Columbia	MD	N/A	N/A
Elson; Clive	Halifax	N/A	N/A	CAX
Taylor, Jr.; John R.	New York	NY	N/A	N/A

US-CL-CURRENT: 514/55; 514/2, 536/20

Full	Title	Citation	Front	Review	Classification	Date	Reference	Claims	KWIC	Draw Desc	Image
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☐ 4. Document ID: US 5885829 A

L8: Entry 4 of 21

File: USPT

Mar 23, 1999

US-PAT-NO: 5885829

DOCUMENT-IDENTIFIER: US 5885829 A

TITLE: Engineering oral tissues

DATE-ISSUED: March 23, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Mooney; David J.	Ann Arbor	MI	N/A	N/A
Rutherford; Robert B.	Ann Arbor	MI	N/A	N/A

US-CL-CURRENT: 435/325; 424/422, 424/435, 424/49, 435/374, 435/378, 435/69.1

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KWIC	Draw Desc	Image
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☐ 5. Document ID: US 5871724 A

L8: Entry 5 of 21

File: USPT

Feb 16, 1999

US-PAT-NO: 5871724

DOCUMENT-IDENTIFIER: US 5871724 A

TITLE: Tissue-derived tumor growth inhibitors, methods of preparation and uses thereof

DATE-ISSUED: February 16, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Iwata; Kenneth K.	Westbury	NY	N/A	N/A
Foulkes; J. Gordon	Huntington	NY	N/A	N/A
Dijke; Peter Ten	Uppsalla	N/A	N/A	SEX
Haley; John D.	Great Neck	NY	N/A	N/A

US-CL-CURRENT: 424/85.1; 424/85.2, 514/12, 514/2

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KWIC	Draw Desc	Image
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☐ 6. Document ID: US 5833988 A

L8: Entry 6 of 21

File: USPT

Nov 10, 1998

US-PAT-NO: 5833988

DOCUMENT-IDENTIFIER: US 5833988 A

TITLE: Transferrin receptor specific antibody-neuropharmaceutical or diagnostic agent conjugates

DATE-ISSUED: November 10, 1998

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Friden; Phillip M.	Bedford	MA	N/A	N/A

US-CL-CURRENT: 424/178.1; 424/143.1, 424/94.1, 514/12, 514/21, 530/350,
530/387.1, 530/389.1, 530/391.1, 530/391.7, 530/391.9, 530/394, 530/399

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KMOC	Draw Desc	Image
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☐ 7. Document ID: US 5824297 A

L8: Entry 7 of 21

File: USPT

Oct 20, 1998

US-PAT-NO: 5824297

DOCUMENT-IDENTIFIER: US 5824297 A

TITLE: Tissue-derived tumor growth inhibitors, methods of preparation and uses thereof

DATE-ISSUED: October 20, 1998

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Iwata; Kenneth K.	Westbury	NY	N/A	N/A
Foulkes; J. Gordon	Huntington	NY	N/A	N/A
Dijke; Peter Ten	Upsala	N/A	N/A	SEX
Haley; John D.	Great Neck	NY	N/A	N/A

US-CL-CURRENT: 424/85.1; 514/12, 514/21, 530/351, 530/399

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KMOC	Draw Desc	Image
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☐ 8. Document ID: US 5814603 A

L8: Entry 8 of 21

File: USPT

Sep 29, 1998

US-PAT-NO: 5814603

DOCUMENT-IDENTIFIER: US 5814603 A

TITLE: Compounds with PTH activity

DATE-ISSUED: September 29, 1998

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Oldenburg; Kevin R.	Fremont	CA	N/A	N/A
Selick; Harold E.	Belmont	CA	N/A	N/A

US-CL-CURRENT: 514/12; 530/399, 530/402

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KWIC	Draw Desc	Image
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☐ 9. Document ID: US 5741776 A

L8: Entry 9 of 21

File: USPT

Apr 21, 1998

US-PAT-NO: 5741776

DOCUMENT-IDENTIFIER: US 5741776 A

TITLE: Method of administration of IGF-I

DATE-ISSUED: April 21, 1998

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Clark; Ross G.	Pacifica	CA	N/A	N/A
Gesundheit; Neil	Palo Alto	CA	N/A	N/A
Hammerman; Marc R.	St. Louis	MO	N/A	N/A
Miller; Steven B.	St. Louis	MO	N/A	N/A

US-CL-CURRENT: 514/12; 514/21, 514/4

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KWIC	Draw Desc	Image
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☐ 10. Document ID: US 5665383 A

L8: Entry 10 of 21

File: USPT

Sep 9, 1997

US-PAT-NO: 5665383

DOCUMENT-IDENTIFIER: US 5665383 A

TITLE: Methods for the preparation of immunostimulating agents for in vivo delivery

DATE-ISSUED: September 9, 1997

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Grinstaff; Mark W.	Pasadena	CA	N/A	N/A
Soon-Shiong; Patrick	Los Angeles	CA	N/A	N/A
Wong; Michael	Champagne	IL	N/A	N/A
Sandford; Paul A.	Los Angeles	CA	N/A	N/A
Suslick; Kenneth S.	Champagne	IL	N/A	N/A
Desai; Neil P.	Los Angeles	CA	N/A	N/A

US-CL-CURRENT: 424/450; 424/451, 424/465, 424/489

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KWIC	Draw Desc	Image
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Term	Documents
BUFFER.DWPI,TDBD,EPAB,JPAB,USPT,PGPB.	445593
BUFFERS.DWPI,TDBD,EPAB,JPAB,USPT,PGPB.	107547
(7 AND BUFFER).USPT,PGPB,JPAB,EPAB,DWPI,TDBD.	21

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Documents, starting with Document:

[11](#)**Display Format:**[CIT](#)[Change Format](#)

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L8: Entry 8 of 21

File: USPT

Sep 29, 1998

DOCUMENT-IDENTIFIER: US 5814603 A
TITLE: Compounds with PTH activity

DEPR:

"Pharmaceutically acceptable salts" refers to the non-toxic alkali metal, alkaline earth metal, and ammonium salts commonly used in the pharmaceutical industry including the sodium, potassium, lithium, calcium, magnesium, barium, ammonium, and protamine zinc salts, which are prepared by methods well known in the art. The term also includes non-toxic acid addition salts, which are generally prepared by reacting the compounds of this invention with a suitable organic or inorganic acid. Representative salts include the hydrochloride, hydrobromide, sulfate, bisulfate, acetate, oxalate, valerate, oleate, laurate, borate, benzoate, lactate, phosphate, tosylate, citrate, maleate, fumarate, succinate, tartrate, napsylate, and the like.

DEPR:

More generally, this method will be applicable with any enzyme capable of post-translational modification of a protein and can result in either the introduction of positive charge or the deletion of negative charge. Examples of these enzymes include, but are not limited to, those enzymes responsible for the following amino acid modifications: hydroxylation of proline and lysine residues to form hydroxylproline and hydroxylysine; phosphorylation of serine to phosphoserine, carboxylation of glutamate to gamma-carboxyglutamate; the introduction of amide groups to C-terminal residues, e.g., glycynamide; the methylation, acetylation or phosphorylation of the epsilon-amino group of lysine; glycosylation; and the attachment of prosthetic groups, e.g., the attachment of carbohydrates to glycoproteins.

DEPR:

Generally, buffers will also be incorporated into the reservoir to maintain the reservoir environment at the same charge as the electrode. Typically, to minimize competition for the electric current, a buffer having the opposite charge to the drug will be employed. In some situations, for example, when the appropriate salt is used, the drug may act as its own buffer. Other variables which may effect the rate of transport include drug concentration, buffer concentration, ionic strength, nonaqueous cosolvents, and any other constituents in the formulation. However, as discussed above, to achieve the highest transport efficiency, the concentration of all ionic species, save the pharmaceutical agent itself, is minimized.

DEPR:

The drug is administered through an electrode having the same charge as the drug, and a return electrode opposite in charge to the drug is placed at a neutral site on the body surface. The operator then selects a current intensity below the pain threshold level of the patient and allows the current to flow for an appropriate length of time. Ions transferred through the skin are taken up by the micro-circulation at the dermal-epidermal junction, while the current proceeds through the skin tissues to the return electrode. The current intensity should be increased slowly, maintained for the length of time of the treatment, and then decreased slowly at the end of the treatment. The current must be within comfortable toleration of the patient, with a current density which is generally less than 0.5 mAmp/cm.² of the electrode surface.

DEPR:

Typically, the iontophoretic carrier solution will also contain other ionic species, in addition to the PTH analog. For example, these ionic species can arise from buffer solutions that may be present to maintain the pH of the solution. As expected from a coulombic mechanism of electrotransport, to achieve the highest transport efficiency, the concentration of all ionic species, save the PTH analog, should be minimized.

DEPR:

Dosage forms for the topical administration of a compound of this invention include powders, sprays, ointments, pastes, creams, lotions, gels, solutions, patches and inhalants. The active compound may be mixed under sterile conditions with a pharmaceutically- acceptable carrier, and with any preservatives, buffers, or propellants which may be required.

DEPR:

For solutions used in making aerosols, the preferred range of concentration of the PTH analog is 0.1-100 milligrams (mg)/milliliter (ml), more preferably 0.1-30 mg/ml, and most preferably, 1-10 mg/ml. Usually the solutions are buffered with a physiologically compatible buffer such as phosphate or bicarbonate. The usual pH range is 5 to 9, preferably 6.5 to 7.8, and more preferably 7.0 to 7.6. Typically, sodium chloride is added to adjust the osmolality to the physiological range, preferably within 10% of isotonic. Formulation of such solutions for creating aerosol inhalants is discussed in Remington's Pharmaceutical Sciences, see also, Ganderton and Jones, Drug Delivery to the Respiratory Tract, Ellis Horwood (1987); Gonda (1990) Critical Reviews in Therapeutic Drug Carrier Systems 6:273-313; and Raeburn et al. (1992) J. Pharmacol. Toxicol. Methods 27:143-159.

DEPR:

The in vitro skin permeation rate of the PTH analogs can be measured using diffusion cells. Human, mouse or porcine skin is placed on the lower half of the diffusion cell with the stratum corneum facing the donor compartment. The donor compartment contains a solution of the pharmaceutical agent and the cathode. The receiver compartment contains a buffer solution and the anode. An electric current is applied and the amount of transported drug can be calculated ($\mu\text{g}/\text{cm}^2 \cdot \text{hr}$). Alternatively, an iontophoresis device containing the pharmaceutical agent to be tested can be placed on the stratum corneum. The receiver compartment again would contain a buffer solution. The device is activated and the amount of transported drug can be calculated ($\mu\text{g}/\text{cm}^2 \cdot \text{hr}$).

DEPR:

Conventional flow-through diffusion cells can also be used to measure the in vitro skin permeation rate of pharmaceutical agents. Typically these cells will have an active area of 1 cm^2 and a receiving volume of 3 ml. The receptor fluid, generally isotonic saline or buffer solution, is pumped into and through the cells, by a peristaltic pump. Samples can be collected in glass vials arranged in an automatic fraction collector. The amount of drug permeating across the skin ($\mu\text{g}/\text{cm}^2 \cdot \text{hr}$) is calculated from the cumulative release.

DEPR:

Plasmid pBAD18 was digested with restriction enzymes NheI and HindIII, and the large DNA fragment resulting from the digestion was gel purified. A piece of synthetic "linker" DNA, described below, was then added to the plasmid pBAD18 DNA at a molar ratio of 3:1 (about 0.75 μg of vector), and the resulting mixture was ligated overnight at 14.degree. C. with T4 DNA ligase. The DNA was then precipitated, resuspended in TE buffer, and "wild" type vector destroyed by digestion with restriction enzyme KpnI. The ligated DNA was then precipitated, resuspended in TE, and electroporated into E. coli cell line DH10B (commercially available from Gibco BRL). The transformed cells were then plated onto LB (Luria-Bertani media) agar plates containing 50 $\mu\text{g}/\text{mL}$ of ampicillin, and after overnight culture at 37.degree. C., about 20 colonies were selected, grown individually for 8 hours in 3 mL of media containing ampicillin, and the plasmid

DNA purified. Restriction analysis of these plasmids indicated that all 20 contained the linker. Four of these plasmids were then sequenced, and all four contained the correct linker sequence. The plasmid that was constructed was designated plasmid pBAD/PepEV.

DEPR:

E. coli DH10B containing the plasmid pBAD/PTH/OC (i.e., the 8-mer coding plasmid) was grown overnight in LB-media containing ampicillin (50 to 100 $\mu\text{g/mL}$). Ten mL of this culture were used to inoculate a 500 mL culture of Superbroth (35 g/L Bacto-tryptone, 20 g/L yeast extract, 5 g/L NaCl, and NaOH to pH=7.5) containing ampicillin. The cells were allowed to grow to an OD_{sub.600} of about 0.5 to 1.0 and L-(+)-arabinose was added to a final concentration of 0.2%. The cells were allowed to grow for an additional 3 hours. At the end of this time, the OD_{sub.600} was between 1.5 to 3. The cells were harvested by centrifugation and washed sequentially with 250 mL of WTEK buffer (50 mM Tris, pH=7.5, 10 mM EDTA, 100 mM KCl); 250 mL of PBS; and 250 mL of 10 mM Tris, pH=7.5. The cells were then resuspended in 100 mL of a solution composed of 10 mM Tris, pH=7.5; 0.1 mg/mL of protease inhibitor N-tosyl-L-phenylalanine chloromethyl ketone (TPCK); 0.1 mg/mL of protease inhibitor N-tosyl-L-lysine chloromethyl ketone (TLCK); 0.1 mg/mL of protease inhibitor phenylmethylsulfonyl fluoride (PMSF); and 0.05 mg/mL lysozyme). The resulting solution was incubated on ice for 1 hour. The cells were then freeze-thawed; 1 mg of DNase was added to the freeze-thawed cells; and the resulting mixture was incubated on ice for an additional hour.

DEPR:

Inclusion bodies from the cells were purified by centrifugation at 10,000.times.g for 15 minutes. The inclusion bodies were solubilized in 10% SDS, but in some cases, sonication of the sample was also necessary to solubilize all of the protein. Binding buffer (5 mM imidazole, 500 mM NaCl, and 20 mM Tris, pH=7.9) was added to dilute the SDS concentration to 1%, and the sample was loaded onto a column containing His-bind resin (Novagen). The column was then washed with 15 column volumes of binding buffer, and bound protein was then eluted with 1 column volume of elution buffer (500 mM NaCl, 100 mM EDTA, and 50 mM Tris, pH=7.9). Two volumes of absolute ethanol were then added to precipitate the protein.

DEPR:

The precipitated PTH polymer was then dissolved in 70% formic acid, and a 500-fold (100 to 1000-fold excess can be used) molar excess of CNBr was added. A time course of cleavage (conducted at different CNBr concentrations to determine the optimal time), as assayed by amino acid analysis, indicated that complete cleavage was achieved in 2 hours at room temperature. After CNBr cleavage, the peptides were lyophilized and resuspended in distilled water. The peptide was purified resuspended in Buffer A (0.1% TFA) and further purified by HPLC using a VYDAC C-18 Hamilton semi-preparative column. Approximately 30 mg of peptide was injected onto the column. The peptide was then eluted with a gradient of 20-40% acetonitrile/0.1% TFA over 40 minutes. The major peak, eluting at approximately 15 minutes, was collected and lyophilized to dryness. Analysis of this peptide by SDS-PAGE and IEF indicated a single species of approximately 4000 daltons with an isoelectric point of approximately 8.7. Further analysis by analytical HPLC on a Vydac C18 column, by capillary zonal electrophoresis, and by amino acid analysis indicated that the peptide was greater than 95% pure.

DEPR:

The UMR106 cells were plated at 1.2.times.10⁵ per well in a 48 well dish. The media (DMEM with fetal calf serum) was removed, and 1 mL of fresh media was applied. PTH (recombinant or synthetic) at various concentrations and 3-isobutyl-1-methyl xanthine (IBMX) at 1 mM final concentration were then added to each well of the plate, which was then incubated for 5 minutes at room temperature. The media was then removed and the cells quickly washed with PBS. The cells were then extracted three times with 1 mL of absolute ethanol. The three extractions were combined and the ethanol removed by evaporation in a "speed vac" centrifuge. The extract was then redissolved in 0.5 mL of buffer (0.05M NaAcetate, pH=5.8, and 0.01% azide). The cAMP concentration was

determined by scintillation proximity assay using a kit available from Amersham. This data indicated that the recombinant PTH analog is approximately 2-to-10-fold more active than the synthetic human PTH.

DEPL:

To prepare the linker that was used in the construction of plasmid pBAD/PepEV, equal amounts (about 0.25 .mu.g) of each oligonucleotide in TE buffer (10 mM Tris, 1 mM EDTA, pH=8.0), were mixed, heated to 95.degree. C. for 2 minutes, and then allowed to cool slowly to room temperature. Restriction enzyme digestion buffer was then added, and the DNA was then treated with restriction enzymes NheI and HindIII.

DEPL:

The two oligonucleotides (about 0.25 .mu.g of each) were mixed together and heated to 95.degree. C.; then, TAq polymerase was added, and the oligonucleotides were annealed at 60.degree. C. Extension of the oligonucleotides to form double-stranded DNA was performed at 72.degree. C. The double-stranded DNA was then precipitated with isopropanol and sodium acetate and resuspended in sterile deionized water. 10X restriction enzyme buffer was added to the mixture, and the DNA was treated with restriction enzymes XhoI and SalI for 2 hours at 37.degree. C. The DNA was then precipitated as above and resuspended in sterile deionized water.

ORPL:

Linkhart et al., 1991, Endo. 128 (3): 1511-1518 Differential regulation of insulin-like growth factor-I (IGF-I) and IGF-II release from cultured neonatal mouse calvaria by parathyroid hormone, transforming growth factor-b, and 1,25-Dihydroxyvitamin D3.

ORPL:

Linkhart et al., 1991, Endo. 125 (3): 1484-1491 Parathyroid hormone stimulates release of insulin()like growth factor-I (IGIF) and IGF-II from neonatal mouse calvaria in organ culture*.

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L8: Entry 9 of 21

File: USPT

Apr 21, 1998

DOCUMENT-IDENTIFIER: US 5741776 A

TITLE: Method of administration of IGF-I**ABPL:**

A method is disclosed that comprises administering insulin-like growth factor-I (IGF-I) to a mammal so as to sustain its biological activity in the mammal comprising administering a therapeutically effective amount of IGF-I to the mammal to provide an exposure to IGF-I for a period of time that stimulates the maximum biological response in the mammal, then discontinuing said administration for a period of time equal to or less than the time period used for administration, and repeating this pattern of administration and discontinuance of administration for a period as long as necessary to achieve or maintain the desired biological response in the mammal.

BSPR:

This invention relates to a method of dosing and administering IGF-I in an intermittent fashion such that the maximum favorable biological activity of IGF-I is achieved and maintained in the treatment of chronic disorders, with minimized unfavorable side effects.

BSPR:

Human insulin-like growth factor-I (IGF-I) is a 7649-dalton polypeptide with a pI of 8.4 [Rinderknecht and Humbel, *Proc. Natl. Acad. Sci. USA*, 73:2365 (1976); Rinderknecht and Humbel, *J. Biol. Chem.*, 253: 2769 (1978)] belonging to a family of somatomedins with insulin-like and mitogenic biological activities that modulate the action of growth hormone (GH). Van Wyk et al., *Recent Prog. Horm. Res.*, 30: 259 (1974); Binoux, *Ann. Endocrinol.*, 41: 157 (1980); Clemmons and Van Wyk, *Handbook Exp. Pharmacol.*, 57: 161 (1981); Baxter, *Adv. Clin. Chem.*, 25: 49 (1986); U.S. Pat. No. 4,988,675; WO 91/03253; and WO 93/23071. IGF-I has hypoglycemic effects similar to insulin but also promotes positive nitrogen balance. Underwood et al., *Hormone Res.*, 24: 166 (1986); Guler et al., *N. Engl. J. Med.*, 317: 137 (1987). Due to this range of activities, IGF-I is being tested in humans for uses ranging from wound healing to the reversal of whole body catabolic states to treatment of heart conditions such as congestive heart failure. Guler et al., *Proc. Natl. Acad. Sci. USA*, 85: 4889 (1988); Duerr et al., *J. Clin. Invest.*, 95: 619-627 (1995). IGF-I is also being tested in the clinic for treating diabetes.

BSPR:

U.S. Pat. Nos. 5,273,961; 5,126,324; 5,187,151; 5,202,119; 5,374,620; 5,106,832; 4,988,675; 5,106,832; 5,068,224; 5,093,317; and 4,876,242 and WO 92/11865 and WO 94/16722 disclose various methods of treating patients using IGF-I.

BSPR:

A general scheme for the etiology of some clinical phenotypes which give rise to insulin resistance and the possible effects of administration of IGF-I on selected representative subjects is given in several references. See, e.g., Elahi et al., "Hemodynamic and metabolic responses to human insulin-like growth factor-1 (IGF-1) in men," in *Modern Concepts of Insulin-Like Growth Factors*, (Spencer, E M, ed.), Elsevier, New York, pp. 219-224 (1991); Quinn et al., *N. Engl. J. Med.*, 323: 1425-1426 (1990); Schalch et al., "Short-term metabolic effects of recombinant human insulin-like growth factor 1 (rhIGF-1) in type 11 diabetes mellitus," in: *Modern Concepts of Insulin-Like Growth Factors*,

(Spencer, E M, ed.), Elsevier, New York, pp. 705-714 (1991); Schoenle et al., *Diabetologia*, 34: 675-679 (1991); Usala et al., *N. Engl. J. Med.*, 327: 853-857 (1992); Lieherman et al., *J. Clin. Endo. Metab.*, 75: 30-36 (1992); Zenobi et al., *J. Clin. Invest.*, 90: 2234-2241 (1992); Zenobi et al., *J. Clin. Invest.*, 89: 1908-1913 (1992); Kerr et al., *J. Clin. Invest.*, 91: 141-147 (1993); and U.S. Pat. No. 4,988,675. WO 94/16722 discloses a method of chronic modification of cell barrier properties by exposing a cell to a modification-effective amount of IGF-I for at least about seven days and a method of chronic amelioration or reversal of insulin resistance. However, when IGF-I was used to treat type II diabetes patients in the clinic at a dose of 120-160 .mu.g/kg twice daily, the side effects outweighed the benefit of the treatment. Jabri et al., *Diabetes*, 43: 369-374 (1994). See also Wilton, *Acta Paediatr.*, 383: 137-141 (1992) regarding side effects observed upon treatment of patients with IGF-I.

BSPR:

IGF-I has also been found to exert a variety of actions in the kidney. Hammerman and Miller, *Am. J. Physiol.*, 265:F1-F14 (1993). It has been recognized for decades that the increase in kidney size observed in patients with acromegaly is accompanied by a significant enhancement of glomerular filtration rate. O'Shea and Layish, *J. Am. Soc. Nephrol.*, 3: 157-161 (1992). U.S. Pat. No. 5,273,961 discloses a method for prophylactic treatment of mammals at risk for acute renal failure. Infusion of the peptide in humans with normal renal function increases glomerular filtration rate and renal plasma flow. Guler et al., *Acta Endocrinol.*, 121: 101-106 (1989); Guler et al., *Proc. Natl. Acad. Sci. USA*, 86: 2868-2872 (1989); Hirschberg et al., *Kidney Int.*, 43: 387-397 (1993); U.S. Pat. No. 5,106,832. Further, humans with moderately reduced renal function respond to short-term (four days) IGF-I administration by increasing their rates of glomerular filtration and renal plasma flow. Hence, IGF-I is a potential therapeutic agent in the setting of chronic renal failure. O'Shea et al., *Am. J. Physiol.*, 264: F917-F922 (1993).

BSPR:

Additionally, renal function can be enhanced over a period of days by the administration of IGF-I in the setting of end-stage chronic renal failure. This is important, since end-stage chronic renal failure is a condition that can only be treated with dialysis or transplantation and the incidence thereof is rapidly increasing. Diabetics and the elderly tend to have this condition. Approximately sixty percent of patients with end-stage chronic renal failure are on hemodialysis, about ten percent are on peritoneal dialysis, and the remaining about thirty percent receive a transplant. Dialysis therapy is initiated in over 50,000 patients each year in the United States. An additional 25% of patients who have reached end-stage renal failure are denied access to dialysis each year. The cost of caring for these patients on dialysis currently averages over \$200 million a month. Furthermore, the patients exhibit an impaired lifestyle on dialysis. Despite the fact that IGF-I can enhance renal function for those experiencing end-stage chronic renal failure, the enhancements of the glomerular filtration rate and renal plasma flow induced by IGF-I short-term do not persist during long-term administration and incidence of side-effects is high. Miller et al., *Kidney International*, 46: 201-207 (1994).

BSPR:

The dynamics of IGF-I interaction with sensitive tissues are complex and incompletely understood. Biological activity of circulating IGF-I is regulated by levels of plasma IGFBPs, which both enhance and inhibit IGF-I actions. Cohick and Clemmons, *Annu. Rev. Physiol.*, 55: 131-153 (1993); Kupfer et al., *J. Clin. Invest.*, 91: 391-396 (1993). In addition, IGFBPs present in tissues regulate the interaction of circulating IGF-I with its receptor. Tissue IGF-I receptor density is altered by changes in levels of circulating IGF-I. In kidney, the numbers of IGF-I receptors are inversely related to levels of circulating IGF-I. Hise et al., *Clin. Sci.*, 83: 223-239 (1991).

BSPR:

It is known that under some circumstances elevated levels of circulating IGF-I are associated with or directly causative of long-term changes in renal function. For example, the enhancements of inulin and PAH clearances that

accompany the elevations of circulating GH and IGF-I in patients with acromegaly are sustained over years of time. Ikkos et al., Acta Endocrinol., 21: 226-236 (1956). An increase in creatinine clearance occurred within the first 12 days of IGF-I administration to a GH-insensitive Laron dwarf. The increase was progressive over the next 59 days. Walker et al., J. Pediatr., 121: 641-646 (1992).

BSPR:

GH stimulates the synthesis of IGFBP3 in liver. Hammerman and Miller, supra; Cohick and Clemmons, supra; Kupfer et al., supra. It is the reduction in levels of circulating GH resulting from IGF-I inhibition of pituitary GH release that is thought to result in the fall of circulating IGFBP3 in humans administered IGF-I. Because of their GH insensitivity, IGFBP3 levels are low and are increased by IGF-I in Laron dwarfs. Kenety et al., Acta Endocrinol., 128: 144-149 (1993). This difference or another in the IGF-I effector system could explain the absence of refractoriness to IGF-I in these individuals.

BSPR:

Walker et al., supra, found that IGF-I increased urinary calcium excretion or urinary volume. Miller et al., supra, did not see such effect. IGF-I also enhances the transport of phosphate across the proximal tubular brush border membrane. Quigley and Baum, J. Clin. Invest., 88: 368-374 (1991). Patients with long-standing acromegaly showed marked renal hypertrophy and had supranormal glomerular filtration rates, suggesting that the hyperfiltration that accompanies long-standing elevations of circulating GH and IGF-I in humans is not injurious to the kidney. Ikkos et al., supra; Hoogenberg et al., Acta Endocrinol., 129: 151-157 (1993).

BSPR:

For complete reviews of the effect of IGF-I on the kidney, see, e.g., Hammerman and Miller, Am. J. Physiol., 265: F1-F14 (1993) and Hammerman and Miller, J. Am. Soc. Nephrol., 5: 1-11 (1994).

BSPR:

As to anabolic indications for IGF-I, in HIV-infected patients treated consecutively with IGF-I, the IGF-I promoted anabolism but tachyphylaxis developed rapidly in the patients. Lieberman et al., U.S. Endocrine Meeting, June 1993 (Abst. 1664), disclosed more fully by Lieberman et al., J. Clin. Endo. Metab., 78: 404-410 (1994). In patients with severe head injuries, a condition associated with profound hypercatabolism and nitrogen loss, infusion of IGF-I produced only a transient positive nitrogen balance. In the first week the patients experienced a positive nitrogen balance, but during the second week, a negative nitrogen balance developed. Chen et al., U.S. Endocrine Meeting, June 1993 (Abst. 1596).

BSPR:

All of these studies indicate that there is a need in the art for a treatment with IGF-I that does not subside after a certain period of treatment time and one that both maximizes efficacy and minimizes the side effects of IGF-I.

BSPR:

Accordingly, this invention supplies a method for administering IGF-I to a mammal in an intermittent fashion so as to sustain its biological response in the treatment of a chronic disorder in the mammal comprising administering a therapeutically effective amount of IGF-I to the mammal to provide an exposure to IGF-I for a period of time that provides the maximum biological response in the mammal, then discontinuing said administration for a period of time equal to or less than the time period during which the IGF-I was previously administered, then administering a therapeutically effective amount of IGF-I to the mammal to provide an exposure to IGF-I for a period of time that provides the maximum biological response in the mammal, then discontinuing said administration for a period of time equal to or less than the time period during which the IGF-I was just previously administered, and repeating this pattern of administration and discontinuance of administration for as long as necessary to achieve or maintain sustained biological response in the mammal.

BSPR:

In another aspect, this invention supplies a method for treating chronic renal failure in a mammal by treating with IGF-I in an intermittent fashion comprising administering a therapeutically effective amount of IGF-I to the mammal to provide an exposure to IGF-I for from about three to twelve days, then discontinuing said administration for from about two to seven days, then administering a therapeutically effective amount of IGF-I to the mammal to provide an exposure to IGF-I for from about three to twelve days, then discontinuing said administration for from about two to seven days, and repeating this pattern of administration and discontinuance of administration for as long as necessary to achieve or maintain sustained renal function in the mammal, said time periods of discontinuing administration being for a period of time equal to or less than the time period during which the IGF-I was just previously administered.

DRPR:

FIG. 3 depicts IGF-I levels for five patients as a function of days in the study (normal is 135-449 ng/mL).

DEPR:

As used herein, "biological response" refers to the favorable response of a mammal having a specific chronic disorder to treatment with IGF-I. Maximizing the response means improving efficacy but at the same time avoiding or at least minimizing the occurrence of side effects. The response is tailored to the disorder being treated. Thus, for example, "biological response" in the context of end-stage chronic renal failure, or "improved kidney function" refers to delaying the time a patient has to go on dialysis by at least one month. "Biological response" in the context of diabetic indications refers to decreasing a mammal's blood sugar levels, and reducing markers of glycemic control such as hemoglobin A1c. In the context of anabolic disorders, the phrase refers to increasing anabolic parameters and clinical phenotypes. In the context of cardiac disorders, the phrase refers to improvement in cardiac function. "Sustained biological response" refers to maintaining the biological response (without substantial side effects) for an extended period of time as is considered appropriate for the disorder in question.

DEPR:

"Provide an exposure to IGF-I" refers to an exposure that is continuous or in consecutive days (at least once a day consecutively) over the period of days specified. This may be accomplished, for example, by daily or twice daily injections, or by long-acting formulations of IGF-I that are taken once but provide continuous exposure of the mammal to the IGF-I for the duration of the desired exposure period of the treatment. The discontinuance of treatment for injections of at least daily frequency is a continual or daily consecutive lack of treatment for the period specified. The discontinuance of treatment for long-acting formulations begins when the blood levels of IGF-I have fallen below the desired or maximum level for efficacious treatment and ends when the IGF-I is again administered to the mammal.

DEPR:

One method of judging the correct intermittent regimen for a particular indication of use is to administer IGF-I by daily injection until the time of the maximal effect of the drug is obtained and before the biological response wanes or until side effects occur to any significant degree, whichever occurs earlier. This is the most suitable period of treatment as defined herein. The maximum period of rest from treatment will be an equivalent, or more usually a lesser, interval of time. Hence, the two gauges of the intermittent regime that is most appropriate are the maximum efficacy observed and the occurrence of side effects, with the treatment being tailored to minimize the side effects seen in a particular disease state.

DEPR:

"Side effects" refer to effects of treatment with IGF-I that are not the intended effects such as, e.g., cardiac effects including syncopal episodes with

lightheadedness, fainting and seizure activity, bradycardia associated with nausea and dizziness, transient atrial fibrillation, hypoglycemia, and asymptomatic tachycardia. Other side effects include Bell's palsy, intracranial hypertension, papilledema, hypokalemia, mild weight gain, dyspnea, bilateral jaw tenderness, orthostatic hypotension, local burning or pain at the injection site, headaches including migraine headaches, abdominal pain, sinusitis, jaw pain, edema, paratid swelling, myalgias, arthralgias, fatigue, weakness, snoring, breast enlargement, back pain, flushing, difficulty breathing, transient hypertension, kidney stones, and coarsening of facial features. Effects on breathing include tonsillar and adenoidal enlargement and acute pulmonary edema, and adverse effects on blood cells include thrombocytopenic purpura and neutropenia.

DEPR:

As used herein "chronic" refers to a disorder that is not acute but rather occurs more or less on a continuous level. A "disorder" is any condition that would benefit from treatment with IGF-I, including but not limited to, for example, chronic lung disease, hyperglycemic disorders as set forth below, chronic renal disorders, such as chronic renal insufficiency, end-stage chronic renal failure, glomerulonephritis, interstitial nephritis, pyelonephritis, glomerulosclerosis, e.g., Kimmelstiel-Wilson in diabetic patients and kidney failure after kidney transplantation, obesity, GH-insufficiency, Turner's syndrome, Laron's syndrome, short stature, undesirable symptoms associated with aging such as increasing lean mass-to-fat ratios, immunological disorders such as immunodeficiencies including decreased CD4 counts and decreased immune tolerance or chemotherapy-induced tissue damage, bone marrow transplantation, diseases or insufficiencies of cardiac structure or function such as chronic heart conditions and congestive heart failure, chronic neuronal, neurological, or neuromuscular disorders, e.g., peripheral neuropathy, multiple sclerosis, myotonic dystrophy, catabolic states associated with wasting caused by any condition, including, e.g., trauma or wounding or infection such as with a bacterium or human virus such as HIV, Laron dwarfism, wounds, skin disorders, gut structure and function that need restoration, and so forth. The disorder being treated may be a combination of two or more of the above disorders.

DEPR:

"Long-acting formulations" of IGF-I refer to formulations that maintain levels of exogenously administered IGF-I in the blood for an extended period of time, generally beyond one day, so that injections need not be given on a daily basis. Long-acting formulations include, for example, sustained-release formulations, IGF-I that is PEGylated, and IGF-I formulated or complexed with an IGF binding protein.

DEPR:

As used herein, IGF-I refers to insulin-like growth factor-I from any species, including bovine, ovine, porcine, equine, avian, and preferably human, in native-sequence or in variant form, and from any source, whether natural, synthetic, or recombinant. IGF-I has been isolated from human serum and produced recombinantly. See, e.g., EP 123,228 and 128,733.

DEPR:

Preferred herein for human use is human native-sequence, mature IGF-I, more preferably without a N-terminal methionine, prepared, e.g., by the process described in EP 230,869 published Aug. 5, 1987; EP 128,733 published Dec. 19, 1984; or EP 288,451 published Oct. 26, 1988. More preferably, this native-sequence IGF-I is recombinantly produced and is available from Genentech, Inc., South San Francisco, Calif. for clinical investigations.

DEPR:

The preferred IGF-I variants are those described in U.S. Pat. No. 5,077,276; WO 87/01038; and WO 89/05822, i.e., those wherein at least the glutamic acid residue is absent at position 3 from the N-terminus of the mature molecule or those having a deletion of up to five amino acids at the N-terminus. The most preferred variant has the first three amino acids from the N-terminus deleted (variously designated as brain IGF, tIGF-I, des(1-3)-IGF-I, or des-IGF-I).

DEPR:

The IGF-I is administered to the mammal by any suitable technique, depending mainly on the nature of the disorder and type of mammal, including parenterally, intranasally, intrapulmonary, orally, or by absorption through the skin. It can be administered locally or systemically. Examples of parenteral administration include subcutaneous, intramuscular, intravenous, intraarterial, and intraperitoneal administration. This includes injections (single or multiple, e.g., 1-4 per day) or infusions. Preferably, the IGF-I is administered by daily or twice daily subcutaneous injections or by subcutaneous or intravenous infusion.

DEPR:

The IGF-I to be used in the therapy will be formulated and dosed in a fashion consistent with good medical practice, taking into account the clinical condition of the individual patient (especially the side effects of treatment with IGF-I as noted above), the site of delivery of the IGF-I composition, the method of administration, the scheduling of administration, and other factors known to practitioners. The incidence of side effects of IGF-I may be reduced by decreasing the dose. Kupfer et al., supra; Hartman et al., J. Clin. Invest., 91: 2453-2462 (1993). The effective amount of IGF-I for purposes herein is thus determined by such considerations and is an amount that increases and maintains the relevant, favorable biological response of the mammal.

DEPR:

As a general proposition, the total pharmaceutically effective amount of IGF-I administered parenterally per dose will be in the range of about 5 to 1000 $\mu\text{g/kg/day}$, preferably 10 to 500 $\mu\text{g/kg/day}$, more preferably about 30 to 200 $\mu\text{g/kg/day}$, of patient body weight, although, as noted above, this will be subject to a great deal of therapeutic discretion. For example, with treatment of chronic renal failure, the dose per day is preferably about 10 to 160 $\mu\text{g/kg}$, more preferably 20 to 100 $\mu\text{g/kg}$, and most preferably about 25 to 75 $\mu\text{g/kg}$.

DEPR:

The period of treatment with IGF-I is that which provides a maximum biological response as defined above, preferably about 3 to 12 days, more preferably 3-8 days, most preferably 4-7 days, and the off-treatment period is about 2-10 days, more preferably 2-8 days, most preferably 2-7 days. In a particularly preferred embodiment, the on-treatment period is about 3-5 days for a 7-day cycle and about 7-12 days for a 14-day cycle, and the off-treatment period is about 2-4 days for a 7-day cycle and about 2-7 days for a 14-day cycle. The specific length of these periods depends mainly on efficacy data, side effects if any, the time required to achieve maximum biological response, the maximum time required for maintenance of a biological response; and, for the off-treatment, the period of treatment employed as noted below. The treatment pattern of administering IGF-I and then stopping administration thereof is repeated until the desired maintained biological response is obtained in the mammal. In one embodiment, the same length of treatment and discontinuance of treatment is repeated and the same dosing is used during each cycle; however, the invention also encompasses the situation where each cycle of on and off treatment involves a different period of time and/or dosing. For example, if side effects were observed during the initial treatment period, the repeated treatment period and/or dosing might be reduced, whereas if efficacy increases during the first treatment with no substantial side effects, the second period of treatment and/or dosing might be increased.

DEPR:

The on- and off-treatment periods of time are proportionate and interdependent, such that the period of time when treatment is not carried out is no longer than the period when treatment is carried out. For example, if the patient is treated for 4 days with IGF-I, he or she can be off treatment for up to 3 days, but not for 4 or more days; at that point the patient needs to be treated again with IGF-I. In a preferred embodiment, the cycle of treatment adds up to a total of 7 days or 14 days to ease tracking of the treatment by the patients. The table

below illustrates this scheme:

DEPR:

One of the purposes of the intermittent therapy with IGF-I is to maintain stable plasma levels of some of the IGF binding proteins to facilitate the most appropriate treatment with IGF-I. Alternatively, the IGF-I may be formulated so as to have a continual presence in the blood during the course of treatment, as for example by being made into a long-acting formulation, e.g., by being covalently attached to a polymer such as polyethylene glycol (PEG). In this embodiment, fewer injections could be given in each course of treatment due to the persistence of the IGF-I in the body by way of the long-acting nature of the preparation. In this embodiment, if each injection causes an exposure of several days, the single injections tailored to give exposures as described above would be the regimen used. If the formulation is long-acting, the injections are suitably given at weekly intervals or at multiples of weekly intervals.

DEPR:

Suitable examples of sustained-release preparations, which are one type of long-acting formulation, include semi-permeable matrices of solid hydrophobic polymers containing the protein, which matrices are in the form of shaped articles, e.g., films, or microcapsules. Examples of sustained-release matrices include polyesters, hydrogels (e.g., poly(2-hydroxyethyl-methacrylate) as described by Langer et al., J. Biomed. Mater. Res., 15: 167-277 [1981] and Langer, Chem. Tech., 12: 98-105 [1982] or poly(vinylalcohol)), polylactides (U.S. Pat. No. 3,773,919, EP 58,481), copolymers of L-glutamic acid and gamma ethyl-L-glutamate (Sidman et al., Biopolymers, 22: 547-556 [1983]), non-degradable ethylene-vinyl acetate (Langer et al., supra), degradable lactic acid-glycolic acid copolymers such as the Lupron Depot.TM. (injectable microspheres composed of lactic acid-glycolic acid copolymer and leuprolide acetate), polylactate polyglycolate (PLGA), and poly-D-(-)-3-hydroxybutyric acid (EP 133,988). The IGF-I also may be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization (for example, hydroxymethylcellulose or gelatin-microcapsules and poly-[methacrylate] microcapsules, respectively), in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules), or in macroemulsions. Such techniques are disclosed in Remington's Pharmaceutical Sciences, supra.

DEPR:

Sustained-release IGF-I compositions also include liposomally entrapped IGF-I. Liposomes containing IGF-I are prepared by methods known per se: DE 3,218,121; Epstein et al., Proc. Natl. Acad. Sci. USA, 82: 3688-3692 (1985); Hwang et al., Proc. Natl. Acad. Sci. USA, 77: 4030-4034 (1980); EP 52,322; EP 36,676; EP 88,046; EP 143,949; EP 142,641; Japanese patent application 83-118008; U.S. Pat. Nos. 4,485,045 and 4,544,545; and EP 102,324. Ordinarily the liposomes are of the small (about 200-800 Angstroms) unilamellar type in which the lipid content is greater than about 30 mol. % cholesterol, the selected proportion being adjusted for the most suitably IGF-I therapy. A specific example of a suitable sustained-release formulation is in EP 647,449.

DEPR:

For parenteral administration, in one embodiment, the IGF-I is formulated generally by mixing it at the desired degree of purity, in a unit dosage injectable form (solution, suspension, or emulsion), with a pharmaceutically acceptable carrier, i.e., one that is non-toxic to recipients at the dosages and concentrations employed and is compatible with other ingredients of the formulation. For example, the formulation preferably does not include oxidizing agents and other compounds that are known to be deleterious to polypeptides.

DEPR:

Generally, the formulations are prepared by contacting the IGF-I uniformly and intimately with liquid carriers or finely divided solid carriers or both. Then, if necessary, the product is shaped into the desired formulation. Preferably the carrier is a parenteral carrier, more preferably a solution that is isotonic with the blood of the recipient. Examples of such carrier vehicles include

water, saline, Ringer s solution, and dextrose solution. Non-aqueous vehicles such as fixed oils and ethyl oleate are also useful herein, as well as liposomes.

DEPR:

The carrier suitably contains minor amounts of additives such as substances that enhance isotonicity and chemical stability. Such materials are non-toxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, succinate, acetic acid, and other organic acids or their salts; antioxidants such as ascorbic acid; low molecular weight (less than about ten residues) polypeptides, e.g., polyarginine or tripeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids, such as glycine, glutamic acid, aspartic acid, or arginine; monosaccharides, disaccharides, and other carbohydrates including cellulose or its derivatives, glucose, mannose, or dextrans; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; counterions such as sodium; and/or non-ionic surfactants such as polysorbates, poloxamers, or PEG.

DEPR:

The IGF-I is typically formulated in such vehicles at a concentration of about 0.1 mg/mL to 100 mg/mL, preferably 1-10 mg/mL, at a pH of about 4.5 to 8. Full-length IGF-I is preferably formulated at a pH about 5-6, and des(1-3)-IGF-I is preferably formulated at a pH about 3.2 to 5. It will be understood that use of certain of the foregoing excipients, carriers, or stabilizers will result in the formation of IGF-I salts.

DEPR:

While the IGF-I can be formulated in any way suitable for administration, the preferred formulation contains about 2-20 mg/mL of IGF-I, about 2-50 mg/mL of an osmolyte, about 1-15 mg/mL of a stabilizer, and a buffered solution at about pH 5-6, more preferably pH about 5-5.5. Preferably, the osmolyte is an inorganic salt at a concentration of about 2-10 mg/mL or a sugar alcohol at a concentration of about 40-50 mg/mL, the stabilizer is benzyl alcohol or phenol, or both, and the buffered solution is an acetic acid salt buffered solution. More preferably, the osmolyte is sodium chloride and the acetic acid salt is sodium acetate. Even more preferably, the amount of IGF-I is about 8-12 mg/mL, the amount of sodium chloride is about 5-6 mg/mL, the amount of benzyl alcohol is about 8-10 mg/mL, the amount of phenol is about 2-3 mg/mL, and the amount of sodium acetate is about 50 mM so that the pH is about 5.4. Additionally, the formulation can contain about 1-5 mg/mL of a surfactant, preferably polysorbate or poloxamer, in an amount of about 1-3 mg/mL. Alternatively, the formulation is suitably IGF-I dissolved at 5 mg/ml in 10 mM citrate buffer and 126 mM NaCl at pH 6.

DEPR:

IGF-I to be used for therapeutic administration is preferably sterile. Sterility is readily accomplished by filtration through sterile filtration membranes (e.g., 0.2 micron membranes). Therapeutic IGF-I composition generally is placed into a container having a sterile access port, for example, an intravenous solution bag or vial having a stopper pierceable by a hypodermic injection needle.

DEPR:

The IGF-I ordinarily will be stored in unit or multi-dose containers, for example, sealed ampoules or vials, as an aqueous solution, or as a lyophilized formulation for reconstitution. As an example of a lyophilized formulation, 10-mL vials are filled with 5 mL of sterile-filtered 1% (w/v) aqueous IGF-I solutions, and the resulting mixture is lyophilized. The infusion solution is prepared by reconstituting the lyophilized IGF-I using bacteriostatic Water-for-Injection.

DEPR:

IGF-I can be administered along with a low-protein diet or with a low-protein diet in conjunction with nutrient supplements such as ketoacid supplements.

DEPR:

In addition, the IGF-I is appropriately administered together with any one or more of its binding proteins, for example, those currently known, i.e., IGFBP1, IGFBP2, IGFBP3, IGFBP4, IGFBP5, or IGFBP6, or with the acid-labile subunit (ALS) of the IGF binding complex. Such proteins may be administered separately or as a complex with the IGF-I. The IGF-I may also be coupled to a receptor or antibody or antibody fragment for administration. The preferred binding protein for IGF-I herein is IGFBP3, which is described in U.S. Pat. No. 5,258,287 and by Martin and Baxter, J. Biol. Chem., 261: 8754-8760 (1986). This glycosylated IGFBP3 protein is an acid-stable component of about 53 Kd on a non-reducing SDS-PAGE gel of a 125-150 Kd glycoprotein complex found in human plasma that carries most of the endogenous IGFs and is also regulated by GH.

DEPR:

The administration of the IGF binding protein with IGF-I may be accomplished by the method described in U.S. Pat. No. 5,187,151. Briefly, the IGF-I and IGFBP are administered in effective amounts by subcutaneous bolus injection in a molar ratio of from about 0.5:1 to about 3:1, preferably about 1:1.

DEPR:

In a further embodiment, depending on the chronic disorder being treated, another drug besides IGF-I or IGFBP is administered in conjunction with the IGF-I. For example, for a renal indication, it may be desirable to administer in conjunction with IGF-I other renally active molecules that promote reabsorption and retention of electrolytes such as, e.g., atrial natriuretic peptide (ANP), ANP analogs, or any variants thereof with or without receptor activity, urodilatin, human B-type natriuretic peptide (BNP), angiotension receptor antagonist, vasopressin and its analogs, and endothelin antagonists such as antibodies or peptide antagonists. One example is BQ-123 (Ihara et al., Life Science, 50: 247-250 [1992]; JP 51-94254A published Aug. 3, 1993; Webb et al., Biochem. Biophys. Res. Comm., 185: 887-892 [1992]), a cyclic pentapeptide that is a potent and specific blocker of endothelin A receptors and blocks only the hypertrophic activity induced by endothelin-1, not CT-1, mouse LIF, or phenylephrine. Another example is the parent compound to BQ-123 described by Ihara et al., Biochim. Biophys. Res. Comm., 178: 132-137 (1991). Further examples include those described in EP 647,236; EP 647,449; EP 633,259 (phenyl-sulfonyl amino-pyrimidine derivatives); EP 601,386 (sulfonamide compounds); U.S. Pat. No. 5,292,740 (phenylsulfonamidopyrimidines); and U.S. Pat. No. 5,270,313 (phenyl-sulfonyl-aminopyrimidine derivatives). In addition, angiotensin-converting enzyme (ACE) inhibitors may be beneficial in conjunction with the IGF-I treatment of renal disorders.

DEPR:

In the treatment of congestive heart failure, ACE inhibitors may be useful together with IGF-I by reducing systemic vascular resistance and relieving circulatory congestion. The ACE inhibitors include but are not limited to those designated by the trademarks Accupril.RTM. (quinapril), Altace.RTM. (ramipril), Capoten.RTM. (captopril), Lorensin.RTM. (benazepril), Monopril.RTM. (fosinopril), Prinivil.RTM. (lisinopril), Vasotec.RTM. (enalapril), and Zestril.RTM. (lisinopril). One example of an ACE inhibitor is that sold under the trademark Capoten.RTM.. Generically referred to as captopril, this ACE inhibitor is designated chemically as 1-[(2S)-3-mercapto-2-methylpropionyl]-L-proline.

DEPR:

As another example of combination therapy, for an anabolic indication, it may be desirable to administer both IGF-I and GH to the mammal, each in effective amounts, or each in amounts that are sub-optimal but when combined are effective. Preferably, such amounts are about 50 to 100 .mu.g/kg/day of IGF-I and about 0.3 mg/kg/week GH. Preferably, if GH and IGF-I are administered together, the administration of both IGF-I and GH is by injection using, e.g., intravenous or subcutaneous means. More preferably, the administration is by subcutaneous injection for both IGF-I and GH, most preferably daily injections.

DEPR:

It is noted that practitioners devising doses of both IGF-I and GH should take into account the known side effects of treatment with these hormones. For GH, the side effects include sodium retention and expansion of extracellular volume (Ikkos et al., Acta Endocrinol. (Copenhagen), 32: 341-361 [1959]; Biglieri et al., J. Clin. Endo. Metab, 21: 361-370 [1961]), as well as hyperinsulinemia and hyperglycemia. The side effects of IGF-I are noted above. Indeed, the combination of IGF-I and GH may lead to a reduction in the unwanted side effects of both agents (e.g., hypoglycemia for IGF-I and hyperinsulinism for GH) and to a restoration of blood levels of GH, the secretion of which is suppressed by IGF-I.

DEPR:

The IGF-I and GH, preferably the full-length IGF-I, may be administered separately or may be formulated together in an appropriate carrier vehicle to form a pharmaceutical composition that preferably does not contain cells. In one embodiment, the buffer used for formulation will depend on whether the composition will be employed immediately upon mixing or stored for later use. If employed immediately after mixing, a mixture of full-length IGF-I and GH can be formulated in mannitol, glycine, and phosphate, pH 7.4. If this mixture is to be stored, it is formulated in a buffer at a pH of about 6, such as citrate, with a surfactant that increases the solubility of the GH at this pH, such as 0.1% polysorbate 20 or poloxamer 188. The final preparation may be a stable liquid or lyophilized solid.

DEPR:

The preferred combined composition comprises IGF-I and GH in a weight ratio of IGF-I:GH of between about 1:1 and 100:1 (w/w), about 0.05-0.3 mM of an osmolyte, about 0.1-10 mg/mL of a stabilizer, about 1-5 mg/mL of a surfactant, and about 5-100 mM of a buffer at about pH 5-6. Preferably, the osmolyte is an inorganic salt and the surfactant is nonionic. More preferably, the inorganic salt is sodium chloride or potassium chloride, the stabilizer is phenol or benzyl alcohol, the surfactant is polysorbate or poloxamer, the buffer is sodium acetate or sodium citrate or both, and the amounts of IGF-I and GH are about 2-20 mg/mL and about 0.2-10 mg/mL, respectively, with the weight ratio of IGF-I:GH being between about 1:1 and 50:1. Even more preferably, the amount of IGF-I is about 5-10 mg/mL, the amount of GH is about 1-5 mg/mL, the weight ratio of IGF-I:GH is about 1:1 to 4:1, the amount of sodium chloride is about 5-7 mg/mL, the amount of phenol is about 0.1-3 mg/mL, the amount of benzyl alcohol is about 6-10 mg/mL, the surfactant is polysorbate in an amount of about 1-3 mg/mL, the amount of sodium acetate is about 2.5-4 mg/mL, and the amount of sodium citrate is about 0.1-1 mg/mL.

DEPR:

Renal function can be enhanced by administration of IGF-I at 100 .mu.g/kg subcutaneously each day in the morning for four days in the setting of end-stage chronic renal failure as established by Miller et al., supra. However, renal function returned to the baseline level at 28 days despite ongoing, continuous treatment with IGF-I. In contrast, Example I shown herein demonstrates ongoing efficacy when intermittent dosing is given in the context of end-stage chronic renal failure.

DEPR:

Patients participated in protocols of 4 to 20 weeks in duration. The first day of the protocols was designated as day 0. On day 0, measurements of blood urea nitrogen (BUN), serum Na.sup.+, K.sup.+, HCO.sub.3.sup.-, IGF-I, IGFBP1, -2, and -3, creatinine, calcium, and phosphate were obtained in addition to inulin and PAH clearances. BUN, serum Na.sup.+, K.sup.+, HCO.sub.3.sup.-, creatinine, calciums phosphate, and glucose levels were measured on an Hitachi 747.TM. autoanalyzer (Boehringer Mannheim, Indianapolis, Ind.). IGF-I and IGFBP levels in serum were measured by Endocrine Sciences (Calabasas Hills, Calif.). Dunnett's multiple comparison procedure (Miller et al., supra) was used for multiple comparisons. Values were considered significantly different if p<0.05 for 2-tailed analysis. The data were analyzed using one-way analysis of variance (ANOVA) (Instat, Graph Pad Software Inc., San Diego, Calif.).

DEPR:

On days 1 through 4 the patients received rhIGF-I dissolved at 5 mg/ml in 10 mM citrate buffer and 126 mM NaCl at pH 6 (provided by Genentech, Inc., South San Francisco, Calif.) at a dose of 50 μ g/kg subcutaneously once per day at 8 AM (QAM). These patients were not treated with IGF-I on days 5 through 7 and then treated again on days 8 through 11 and then not treated on days 12 through 14 and then treated, using this administration/dosing pattern for a period of 4 to 20 weeks. Blood samples were obtained on the first and fourth day, at the beginning and end of each cycle of treatment, of each week for the first four weeks, to determine inulin and PAH clearances and IGF-I, IGFBP1, IGFBP2, and IGFBP3 levels.

DEPR:

FIG. 1 shows the inulin clearance for patients 1-5 for up to 24 days in the study. It can be seen that the inulin clearance progressively increased during the course of the treatment regimen of intermittent IGF-I. FIG. 2 shows the PAH clearance for patients 1-5 for up to 24 days in the study. It can be seen that PAH clearance also increased with the treatment regimen. FIG. 3 shows the IGF-I levels for patients 1-5 for up to 24 days in the study. It can be seen that IGF-I levels rise and fall with the intermittent treatment regimen but that IGF-I levels are maintained with each cycle of treatment. FIG. 4 shows the IGFBP3 levels for patients 1-5 for up to 24 days in the study. It can be seen that these levels generally are stable throughout the 24 days of the study. FIG. 5 shows the IGFBP1 levels for patients 1-5 for up to 24 days in the study. It can be seen that these levels generally rise with the treatment regimen. FIG. 6 shows the IGFBP2 levels for patients 1-5 for up to 24 days in the study. These levels rise and fall with the intermittent treatment regimen. FIGS. 7-10 respectively show the inulin clearance patterns for patients for a period of days and weeks in the study. FIG. 11 demonstrates the mean inulin clearance of the five patients treated for 24 days and the four patients that continue on IGF-I for up to 8 weeks.

DEPR:

IGF-I can be administered safely in an intermittent fashion to patients with symptomatic ESRD. The treatment resulted in a sustained improvement in renal function over a 4-week period with resolution of most uremic symptoms. No adverse side effects were observed in patients receiving the IGF-I in an intermittent mode. The improvement in renal function persisted throughout the administration cycle despite IGF-I levels returning to baseline after each period of intermittent exposure and prior to the next treatment cycle. Administration of IGF-I in an intermittent manner did not suppress IGFBP3 levels over the course of four weeks.

DEPR:

Hence, administration of IGF-I in an intermittent fashion to patients with symptomatic ESRD can delay or obviate the need for dialysis or transplantation.

DEPR:

The loss of lean body mass accompanying AIDS-associated cachexia is refractory to current modes of therapy. Ten subjects with AIDS-associated cachexia with an average weight loss of 15.8 \pm 2.1% (range 8.3-24.8%) were infused intravenously with low (4 μ g/kg/hr) or high (12 μ g/kg/hr) doses of recombinant human IGF-I formulated in citrate buffer as described in Example I for 12 hours each day over a period of 10 days following dietary stabilization. (This translates to an IGF-I dose of 48 or 144 μ g/kg/day, respectively.) Protein turnover (13 C-leucine and 15 N-glycine techniques) was measured as follows: 13 C-leucine was infused on days -1 and 10. Twenty-four-hour infusions of 15 N-glycine were performed on days -2 and 9. Nitrogen balance was measured at baseline and during the final three days of IGF-I administration. Nitrogen retention was followed throughout IGF-I infusion.

DEPR:

Cumulative nitrogen retention was significantly positive during the first seven days of treatment (+9.52 \pm 4.09 g, p<0.05), but the effect was transient.

Nitrogen balance and indices of protein turnover during the final three days of treatment were unchanged compared to baseline. There was a trend toward lower (1.32. \pm .0.82 g/day vs. 3.24. \pm .1.19 g/day) and earlier (median day 6 vs. median day 10) peak nitrogen retention with high-dose than with low-dose IGF-I infusion. With repeated IGF-I administration, pre-infusion IGF-I levels increased (low dose: day 1, 151. \pm .23; day 5, 253. \pm .55; day 10, 249. \pm .51 μ g/L; $p=0.02$; high dose: day 1, 124. \pm .16; day 5, 222. \pm .26; day 10, 179. \pm .28 μ g/L; $p=0.02$), but the increment in circulating IGF-I during infusion decreased (low dose: day 1, 284. \pm .77; day 5, 132. \pm .55; day 10, 64. \pm .7 μ g/L; $p=0.08$; high dose: day 1, 376. \pm .42; day 5, 249. \pm .38; day 10, 256. \pm .38 μ g/L; $p<0.01$), producing a trend toward lower steady-state IGF-I levels. This was accompanied by declining levels of IGFBP3 (day 1, 3.09. \pm .0.21; day 5, 2.77. \pm .0.27; day 10, 2.57. \pm .0.20 mg/L; $p=0.0003$ for all 10 subjects together). Levels of IGFBP1 and IGFBP2 were also significantly affected by repeated IGF-I administration.

DEPR:

Hence, IGF-I promotes anabolism but tachyphylaxis develops rapidly. This is reported by Lieberman et al. (1993 and 1994), supra.

DEPR:

In this study, the utility of IGF-I was examined in immobilized patients with severe head injuries, a condition associated with profound hypercatabolism and nitrogen loss. Within 72 hours of injury, male and female patients ($n=24$, 18-46 years) were randomized into two groups, receiving either an IV infusion of 0.01 mg/kg/hr recombinant human IGF-I in citrate buffer as described in Example I or saline for 14 days. Serum glucose concentrations and nitrogen balance were determined on every day of treatment. The interrelationships of free and total IGF-I with IGFBP2 and -3, and with other hormones, particularly insulin and GH, were established by measuring the plasma concentrations in samples collected prior to, during, and immediately after the infusions. In the majority of patients, free IGF-I was not measurable ($<6.25 \mu$ g/L) at any time point. Glucose and insulin concentrations were comparable between the two groups throughout the study.

DEPR:

In the 11 saline-treated patients, mean (\pm SE) total IGF-I was 93. \pm .13 μ g/L at pre-treatment, and the patients were in negative nitrogen balance (-2.9 g/day nitrogen) during the first week. As the patients recovered, total IGF-I increased to 144. \pm .29 μ g/L by day 14, approaching endogenous levels in healthy adults (about 200 μ g/L). This increase in IGF-I did not occur until day 8 of treatment and was not associated with changes in IGFBP3, IGFBP2, or GH. However, during the second week of treatment, the saline-treated patients lost an average of 5.0 g/day nitrogen. Total IGF-I in the 13 patients on IGF-I increased from 78. \pm .14 to 466. \pm .40 μ g/L by day 2. In contrast to the saline-treated group, the IGF-I-treated patients retained 1.3 g/day nitrogen for the first week. The IGF-I-treated patients only maintained total IGF-I steady state for 2 days; then IGF-I declined to 220. \pm .31 μ g/L by day 14 prior to discontinuing therapy. This decline was associated with a decrease in IGFBP3 from 2.8. \pm .0.2 to 2.1. \pm .0.2 mg/L, and an increase in IGFBP2 from 275. \pm .46 to 678. \pm .145 μ g/L over the 14-day period. Plasma concentrations of GH declined from 2.3. \pm .0.6 to a nadir of 0.4. \pm .0.1 μ g/L on day 10 of IGF-I treatment. Furthermore, the IGF-I-treated patients lost an average of 4.9 g/day nitrogen during the second week.

DEPR:

In conclusion, in this study to examine the utility of IGF-I in critically ill patients, infusion of IGF-I produced only a transient positive nitrogen balance in these patients with severe catabolism. In the first week the patients experienced a positive nitrogen balance, but during the second week, a negative nitrogen balance was observed. Infusion of IGF-I led to the suppression of GH and IGFBP3, and to increases in IGFBP2, resulting in enhanced clearance of total IGF-I. This study is described by Chen et al., supra.

DEPR:

These two studies show that for 7 days of IGF-I treatment an anabolic effect was observed which subsided after 7 days. From these results it would be expected that the maximum period of treatment using IGF-I for anabolic response would be 7 days, followed by a rest from treatment of up to 7 days. Hence, when these two type of patients are treated identically as described, except that after 7 days of treatment with IGF-I they are not treated for from 2 to 7 days, they are expected to sustain a positive anabolic effect without tachyphylaxis.

DEPR:

Seven patients with type II diabetes were injected subcutaneously with a dose of 120-160 $\mu\text{g/kg}$ twice daily with recombinant human IGF-I obtained from Chiron Corp. for 4-52 days, as described by Jabri et al., supra. Four patients exhibited comparable or enhanced, whereas three had diminished, blood glucose control when treated with IGF-I relative to that while treated twice daily with NPH insulin during the six-week control period. The occurrence of adverse side effects in all patients compelled the authors to discontinue IGF-I administration before completing the 8-week treatment period. These adverse effects included edema, mild weight gain, occasional dyspnea, bilateral jaw tenderness, arthralgias and myalgias, fatigue, tachycardia, flushing, orthostatic hypotension, and local burning at the injection site. The authors concluded that the frequency and severity of side effects associated with administering high-dose subcutaneous IGF-I continuously to obese insulin-resistant diabetic patients made it an unacceptable therapeutic agent for these patients despite its ability to produce reasonable blood glucose control in about 50% of them.

DEPR:

It is expected that if the same patients are treated with IGF-I under the same conditions, but using intermittent dosing (3-12 days treatment, 2-10 days off treatment, 3-12 days treatment, etc., where the off-treatment period chosen is no longer than the on-treatment period utilized), their glucose levels will be lowered without the side effects observed.

DETL:

TABLE III

															Day 0	
3	7	10	14	17	21	24										
Inulin	7.7	10.8	10.2	9.8	10.9	13.9	11.4	12.0	Clearance	Std. error	0.8	0.8	0.9	0.7		
0.7	0.9	1.1	0.5	1.1	PAH	48.9	53.4	58.4	51.7	54.8	53.9	54.6	60.6	Clearance	Std. error	10.4
15.2	11.0	12.6	15.4	13.9	13.4	16.6	<u>IGF-I</u>	Level	170.8	291.8	153.5	338.4	144.4	276.5	137.8	303.8
Std. error	22.2	63.3	20.7	71.7	15.0	46.7	7.9	19.9	BP-1	Level	26.3	79.5	79.8	83.8	50.9	77.5
Std. error	6.1	53.7	41.5	40.3	23.7	54.2	29.8	20.7	BP-2	Level	2004.5	2570.5	1803.0	2701.0	1749.8	2256.3
1888.8	2326.4	Std. error	809.1	1160.7	762.9	823.7	606.2	604.3	636.0	551.1	BP-3	Level	3.7	3.5	4.1	4.0
3.6	3.4	3.7	3.8	Std. error	0.2	0.2	0.3	0.2	0.2	0.1	0.4	0.2				

CLPR:

1. A method for administering insulin-like growth factor-I (IGF-I) to a mammal so as to sustain its biological response in the treatment of a chronic disorder in the mammal comprising administering a therapeutically effective amount of IGF-I to the mammal to provide an exposure to IGF-I for a period of time that provides the maximum biological response in the mammal, then discontinuing said administration for a period of time equal to or less than the time period during which the IGF-I was previously administered, then administering a therapeutically effective amount of IGF-I to the mammal to provide an exposure to IGF-I for a period of time that provides the maximum biological response in the mammal, then discontinuing said administration for a period of time equal to or less than the time period during which the IGF-I was just previously administered, and repeating this pattern of administration and discontinuance of administration for as long as necessary to achieve or maintain sustained biological response in the mammal.

CLPR:

7. The method of claim 1 wherein when the IGF-I is administered, it is administered at least once a day consecutively.

CLPR:

8. The method of claim 1 wherein when the IGF-I is administered, it is administered in a long-acting formulation.

CLPR:

15. The method of claim 11 wherein the drug is an IGF-I binding protein or acid-labile subunit.

CLPR:

16. The method of claim 1 wherein the IGF-I is complexed with an IGF binding protein or acid-labile subunit.

CLPR:

17. The method of claim 1 wherein the IGF-I is administered subcutaneously or intravenously.

ORPL:

Chen et al., "Recombinant human IGF-I infusion results in transient improvement in nitrogen balance: evidence for IGF-I autoregulation" US Endocrine Meeting (Abstract 1596) p. 449 (1993).

ORPL:

Elahi et al., "Hemodynamic and metabolic responses to human insulin-like growth factor I (IGF-I) in men" Modern Concepts of Insulin-Like Growth Factors, Spenser, ed., New York:Elsevier Science Publ. Co. pp. 219-224 (1991).

ORPL:

Hammerman, "Ask the expert: What are the clinical uses of IGF I in acute and chronic renal failure?" Ped. Nephrology 8:544 (1994).

ORPL:

Lieberman et al., "Effects of recombinant human insulin-like growth factor-I (rhIGF-I) on total and free IGF-I concentrations, IGF-binding proteins, and glycemic response in humans" J. Clin. Endocrinol. and Metab. 75(1):30-36 (1992).

ORPL:

Miller et al., "Effects of IGF-I on renal function in end-stage chronic renal failure" Kidney Int. 46:201-207 (1994).

ORPL:

O'Shea et al., "Effects of IGF-I on renal function in patients with chronic renal failure" Am. J. Physiol. 264:F917-F922 (1993).

WEST

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L8: Entry 20 of 21

File: USPT

Mar 12, 1996

DOCUMENT-IDENTIFIER: US 5498421 A

TITLE: Composition useful for in vivo delivery of biologics and methods employing same

DEPR:

Particles of biologic substantially completely contained within a polymeric shell, or associated therewith, prepared as described herein, are delivered neat, or optionally as a suspension in a biocompatible medium. This medium may be selected from water, buffered aqueous media, saline, buffered saline, optionally buffered solutions of amino acids, optionally buffered solutions of proteins, optionally buffered solutions of sugars, optionally buffered solutions of carbohydrates, optionally buffered solutions of vitamins, optionally buffered solutions of synthetic polymers, lipid-containing emulsions, and the like.

DEPR:

The resulting suspension is optionally filtered through centricon filters (100 kDa cutoff) and the filtered constructs or microbubbles are resuspended in normal saline or suitable buffer. FIG. 1 shows a schematic of such a construct. The average diameter of these constructs is approximately 2 microns. Particle size distribution, as determined with an Elzone particle counter, is seen to be quite narrow (a gaussian distribution with a mean diameter of about 3 microns is typically observed). The size range of particles obtained by this technique is between 0.1 micron to 20 microns. A preferred size range is 0.5 to 10 microns and the most preferred range is 1 to 5 microns. This size is ideally suited for medical applications, since intravenous or intraarterial injections can be accomplished without risk of small blood vessel blockage and subsequent tissue (ischemia due to oxygen deprivation) damage. For comparison, normal red blood cells are approximately 8 microns in diameter.

DEPR:

Useful for the modification of the polymeric shell are electrophilic PEG derivatives including PEG-imidazoles, succinimidyl succinates, nitrophenyl carbonates, tresylates, and the like; nucleophilic PEG derivatives including PEG-amines, amino acid esters, hydrazides, thiols, and the like. The PEG-modified polymeric shell will be expected to persist in the circulation for longer periods than their unmodified counterparts. The modification of polymeric shell with PEG may be performed before formation of the shell, or following formation thereof. The currently preferred technique is to modify the polymeric shell after formation thereof. Other polymers including dextran, alginates, hydroxyethyl starch, and the like, may be utilized in the modification of the polymeric shell.

DEPR:

In accordance with one embodiment of the present invention, polymeric shells prepared as described above are used for the in vivo delivery of biologics, such as pharmaceutically active agents, diagnostic agents or agents of nutritional value. Examples of pharmacologically active agents contemplated for use in the practice of the present invention include analgesic agents (e.g., acetaminophen, aspirin, ibuprofen, morphine and derivatives thereof, and the like), anesthetic gases (e.g., cyclopropane, enflurane, halothane, isoflurane, methoxyfluorane, nitrous oxide, and the like), anti-asthmatic agents (e.g., azelastine, ketotifen, traxanox, and the like), antibiotics (e.g., neomycin, streptomycin, chloramphenicol, cephalosporin, ampicillin, penicillin, tetracycline, and the

like), anti-depressant agents (e.g., nefopam, oxypertine, imipramine, trazadone, and the like), anti-diabetic agents (e.g., biguanidines, hormones, sulfonylurea derivatives, and the like), anti-fungal agents (e.g., amphotericin B, nystatin, candididin, and the like), anti-hypertensive agents (e.g., propranolol, propafenone, oxyprenolol, nifedipine, reserpine, and the like), steroidal anti-inflammatory agents (e.g., cortisone, hydrocortisone, dexamethasone, prednisolone, prednisone, fluazacort, and the like), non-steroidal anti-inflammatory agents (e.g., indomethacin, ibuprofen, ramifenizone, piroxicam, and the like), anti-neoplastic agents (e.g., adriamycin, cyclophosphamide, actinomycin, bleomycin, duanorubicin, doxorubicin, epirubicin, mitomycin, methotrexate, fluorouracil, carboplatin, carmustine (BCNU), cisplatin, etoposide, interferons, phenesterine, taxol (as used herein, the term "taxol" is intended to include taxol analogs and prodrugs, taxanes, and other taxol-like drugs, e.g., Taxotere, and the like), camptothecin and derivatives thereof (which compounds have great promise for the treatment of colon cancer), vinblastine, vincristine, as well as hormonal anti-neoplastic agents such as estrogens, progestogens, tamoxifen, and the like), anxiolytic agents (e.g., dantrolene, diazepam, and the like), enzymatically active agents (e.g., DNase, ribozymes, and the like), nucleic acid constructs (e.g., IGF-1 encoding sequence, Factor VIII encoding sequence, Factor IX encoding sequence, antisense nucleotide sequences, and the like), immunostimulating agents (i.e., interleukins, interferons, vaccines, and the like), immunosuppressive agents (e.g., cyclosporine (CsA), azathioprine, mizorobine, FK506, prednisone, and the like), physiologically active gases (e.g., air, oxygen, argon, nitrogen, carbon monoxide, carbon dioxide, helium, xenon, nitrous oxide, nitric oxide, nitrogen dioxide, and the like, as well as combinations of any two or more thereof), as well as other pharmacologically active agents, such as cimetidine, mitotane, visadine, halonitrosoureas, anthracyclines, ellipticine, benzocaine, barbiturates, and the like.

DEPR:

Examples of agents of nutritional value contemplated for use in the practice of the present invention include amino acids, sugars, proteins, carbohydrates, fat-soluble vitamins (e.g., vitamins A, D, E, K, and the like) or fat, or combinations of any two or more thereof.

DEPR:

The polymeric shell containing solid, liquid or gas cores of biologic allows for the delivery of high doses of biologic in relatively small volumes. This minimizes patient discomfort at receiving large volumes of fluid and minimizes hospital stay. In addition, the walls of the polymeric shell are generally completely degradable in vivo by proteolytic enzymes (e.g., when the polymer is a protein), resulting in no side effects from the delivery system, as is frequently the case with current formulations.

DEPR:

The polymeric shell containing solid or liquid cores of fluorine-containing composition allows for the directed delivery of high doses of the fluorine-containing composition agent in relatively small volumes. This minimizes patient discomfort at receiving large volumes of fluid.

DEPR:

Diseases that can be targeted by such constructs include diabetes, hepatitis, hemophilia, cystic fibrosis, multiple sclerosis, cancers in general, flu, AIDS, and the like. For example, the gene for insulin-like growth factor (IGF-1) can be encapsulated into protein microcapsule shells for delivery for the treatment of diabetic peripheral neuropathy and cachexia. Genes encoding Factor IX and Factor VIII (useful for the treatment of hemophilia) can be targeted to the liver by encapsulation into protein microcapsule shells of the present invention. Similarly, the gene for the low density lipoprotein (LDL) receptor can be targeted to the liver for treatment of atherosclerosis by encapsulation into protein microcapsule shells of the present invention.

DEPR:

In order to obtain hemoglobin constructs with variable affinities to oxygen

(i.e., variable P.sub.50), the IHC were further reacted with PLP, a known allosteric modulator. A suspension of IHC (obtained as in Example 14) in tris buffer was deoxygenated at 10C under nitrogen. 10 ml of the deoxygenated IHC suspension was taken in each of six separate reaction vessels. Different molar ratios of PLP/Hb were added to each of the vessels. They were 0.1/3.0, 0.75/3.0, 1.5/3.0, 3.0/3.0, 4.2/3.0, 6.0/3.0. After 30 minutes, a tenfold excess of sodium borohydride is added and allowed to reduce the Schiff's base for another 30 minutes. The suspension is then filtered by centrifugation, backwashed 3 times with buffered saline, resuspended in buffered saline and stored at 4.degree. C. This modification targets the amino terminal groups of the b-globin chain in deoxyhemoglobin. In this respect the modification closely mimics the action of 2,3-DPG (which binds at lysine EF6(82)b) in stabilizing the deoxy confirmation.

DEPR:

The IHC were prepared as described in Example 14. Polyethylene glycol of MW10,000 (PEG 10k) was reacted with 1,1'-Carbonyl diimidazole CDI according to techniques available in the literature (Beauchamp et al. Analytical Biochemistry 131: 25-33, 1983). The IHC were suspended in 50mMborate buffer pH 8.0 and PEG-CDI (2 fold molar excess relative to total hemoglobin lysines) was added and the reaction mixture stirred at room temperature for 6 hours. The resulting PEG-IHC were then separated by filtration, washed in saline and resuspended in sterile buffered saline.

DEPL:

All binding experiments were done at 25.degree. C. in Tris-buffer (pH 7.4). The IHC retain their ability to bind oxygen reversibly, as demonstrated by UV-visible spectra of the IHC, which indicates the presence of met-Fe(III), oxy-Fe(II) and deoxy-Fe(II) forms. The IHC can be cycled between the deoxy and oxy states for more than ten cycles without substantial degradation. This is important because it indicates that the environment surrounding the active heme site has not been altered significantly in the process of making the IHC red blood cell substitute.

CLPR:

11. A composition according to claim 3 wherein said agent of nutritional value is selected from amino acids, proteins, nucleic acids, sugars, carbohydrates, lipid-soluble vitamins, lipids, or combinations of any two or more thereof.

CLPR:

25. A composition according to claim 1 wherein said polymeric shell containing biologic is suspended in a biocompatible medium, and wherein said biocompatible medium is selected from water, buffered aqueous media, saline, buffered saline, solutions of amino acids, solutions of proteins, solutions of sugars, solutions of vitamins, solutions of carbohydrates, solutions of synthetic polymers, lipid-containing emulsions, or combinations of any two or more thereof.

WEST**End of Result Set**

Generate Collection

L8: Entry 21 of 21

File: USPT

Jan 26, 1993

DOCUMENT-IDENTIFIER: US 5182107 A

TITLE: Transferrin receptor specific antibody-neuropharmaceutical or diagnostic agent conjugates

DEPR:

The neuropharmaceutical agent can be an agent having a therapeutic or prophylactic effect on a neurological disorder or any condition which affects biological functioning of the central nervous system. Examples of neurological disorders include cancer (e.g. brain tumors), Autoimmune Deficiency Syndrome (AIDS), stroke, epilepsy, Parkinson's disease, multiple sclerosis, neurodegenerative disease, trauma, depression, Alzheimer's disease, migraine, pain, or a seizure disorder. Classes of neuropharmaceutical agents which can be used in this invention include proteins, antibiotics, adrenergic agents, anticonvulsants, small molecules, nucleotide analogs, chemotherapeutic agents, anti-trauma agents, peptides and other classes of agents used to treat or prevent a neurological disorder. Examples of proteins include CD4 and superoxide dismutase (including soluble portions thereof), growth factors (e.g. nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), ciliary neurotrophic factor (CNTF), neurotrophins 3,4 and 5 (NT-3,4 and 5) or fibroblast growth factor (FGF)), lymphokines or cytokines (e.g. interferon or interleukins (IL-2)) or antagonists thereof, dopamine decarboxylase and tricosanthin. Examples of antibiotics include amphotericin B, gentamycin sulfate, and pyrimethamine. Examples of adrenergic agents (including blockers) include dopamine and atenolol. Examples of chemotherapeutic agents include adriamycin, methotrexate, cyclophosphamide, etoposide, and carboplatin. An example of an anticonvulsant which can be used is valproate and an anti-trauma agent which can be used is superoxide dismutase. Examples of peptides would be somatostatin analogues and enkephalinase inhibitors. Nucleotide analogs which can be used include azidothymidine (hereinafter AZT), dideoxyinosine (ddI) and dideoxycytidine (ddC).

DEPR:

Periodate coupling requires the presence of oligosaccharide groups on either the carrier or the protein to be delivered. If these groups are available on the protein to be delivered (as in the case of horseradish peroxidase (HRP)), an active aldehyde is formed on the protein to be delivered which can react with an amino group on the carrier. It is also possible to form active aldehyde groups from the carbohydrate groups present on antibody molecules. These groups can then be reacted with amino groups on the protein to be delivered generating a stable conjugate. Alternatively, the periodate oxidized antibody can be reacted with a hydrazide derivative of a protein to be delivered which will also yield a stable conjugate.

DEPR:

Conjugates between ligands and therapeutic or diagnostic agents can also be prepared where the ligands are reactive with other receptors, besides the transferrin receptor, which can also mediate the endocytotic or transcytotic process of transporting macromolecules across the blood-brain barrier. These receptors are also on the cell surface of the endothelial cells which line brain capillaries. Among the receptor types are those that react with insulin-like growth factors 1 or 2 (IGF 1 or 2) or insulin itself. The ligands are those substances which usually react with these receptors (e.g. IGF 1, IGF 2 or

insulin), derivatives of these substances which retain receptor-binding activity or antibodies to these receptors. The therapeutic or diagnostic agents which can be conjugated to the ligands include the above-mentioned proteins such as nerve growth factor, superoxide dismutase, CD-4 or anti-amyloid antibody and drugs such as adriamycin, methotrexate or AZT.

DEPR:

Two murine monoclonal antibodies, B3/25 and T58/30, described by Trowbridge (U.S. Pat. No. 4,434,156 issued Feb. 28, 1984, and Nature Vol. 294, pp. 171-173 (1981)), the contents of both are hereby incorporated by reference, which recognize the human transferrin receptor were tested for their ability to bind to human brain capillary endothelial cells. Hybridoma cell lines which produce B3/25 and T58/30 antibodies were obtained from the American Type Culture Collection (ATCC) in Rockville, Md., and grown in DMEM medium supplemented with 2.0 mM glutamine, 10.0 mM HEPES (pH 7.2), 100 .mu.M nonessential amino acids and 10% heat-inactivated fetal calf serum. The hybridoma cultures were scaled-up in 225 cm.^{sup}2 T-flasks for the production of milligram quantities of IgG antibody. The hybridoma supernatants were concentrated 50.times. using vacuum dialysis and applied to a protein-A sepharose column using the BioRad MAPS buffer system. Purified antibody was eluted from the column, dialyzed against 0.1M sodium phosphate (pH 8.0), concentrated and stored in aliquots at -20.degree. C.

DEPR:

The antibody (10 mg) was first dialyzed overnight against 0.01M sodium bicarbonate (pH 9.0). The HRP (10 mg) was dissolved in 2.5 ml deionized water, 0.1M sodium periodate (160 .mu.l) was added and the mixture was incubated for five minutes at room temperature. Ethylene glycol (250 .mu.l) was added to the HRP solution followed by an additional five minute incubation. This solution was then dialyzed overnight against 1.0 mM sodium acetate buffer (pH 4.4). To the dialyzed OX-26 antibody (2.0 ml, 5.08 mg/ml) was added 200 .mu.l of 1.0M sodium bicarbonate buffer, pH 9.5 and 1.25 ml of the dialyzed HRP solution. This mixture was incubated in the dark for two hours followed by the addition of 100 .mu.l of 10 mg/ml sodium borohydride. The resulting mixture was incubated two additional hours in the dark at 4.degree. C. The protein was precipitated from the solution by the addition of an equal volume of saturated ammonium sulfate and resuspended in a minimal volume of water. Free antibody was removed from the mixture by chromatography on a concanavalin A-sepharose column (a column which binds HRP and the HRP-antibody conjugate and allows the free antibody to pass through). The free HRP was removed by chromatography on a protein A-sepharose column which retains the antibody-HRP conjugate. The final product had an HRP/antibody ratio of 4/1.

DEPR:

To conjugate adriamycin to the antibody, the drug (10 mg in 0.5 ml DPBS) was oxidized by the addition of 200 .mu.l of 0.1M sodium periodate. This mixture was incubated for one hour at room temperature in the dark. The reaction was quenched by the addition of 200 .mu.l of ethylene glycol followed by a five minute incubation. The OX-26 antibody (5.0 mg in 0.5 ml of carbonate buffer (pH 9.5)) was added to the oxidized adriamycin and incubated at room temperature for one hour. Sodium borohydride (100 .mu.l of 10 mg/ml) was added and the mixture was incubated for an additional two hours at room temperature. The free adriamycin was separated from the OX-26 antibody-adriamycin conjugate by chromatography on a PD-10 column. The adriamycin/OX-26 antibody ratio within the conjugate was 2/1. for this particular batch of conjugate.

DEPR:

To demonstrate that anti-transferrin receptor antibody accumulates in the brain parenchyma, homogenates of brains taken from animals injected with labelled OX-26 were depleted of capillaries by centrifugation through dextran to yield a brain tissue supernatant and a capillary pellet. Capillary depletion experiments followed the procedure of Triguero, et al., J. of Neurochemistry, 54: 1882-1888 (1990), hereby incorporated by reference. As for the brain uptake experiments of Example 8, the radiolabelled compounds were injected as a 400 .mu.l bolus into the tail vein of females Sprague-Dawley rats (100-125 gm) under Halothane

anesthesia and the animals were sacrificed at the appropriate time post-injection using a lethal dose of anesthetic. A .sup.3 H-labelled IgG 2a control antibody was co-injected with the .sup.14 C-labelled OX-26 to serve as a control for non-specific radioactivity in the brain due to residual blood. After sacrifice, the brains were removed and kept on ice. After an initial mincing, the brains were homogenized by hand (8-10 strokes) in 3.5 ml of ice cold physiologic buffer (100 mM NaCl, 4.7 mM KCl, 2.5 mM CaCl.sub.2, 1.2 mM KH.sub.2 PO.sub.4, 1.2 mM MgSO.sub.4, 14.5 mM HEPES, 10 mM D-glucose, pH 7.4). Four ml of 26% dextran solution in buffer was added and homogenization was continued (3 strokes). After removing an aliquot of the homogenate, the remainder was spun at 7200 rpm in a swinging bucket rotor. The resulting supernatant was carefully removed from the capillary pellet. The entire capillary pellet and aliquots of the homogenate and supernatant were incubated overnight with 2 ml of Soluene 350 prior to liquid scintillation counting. This method removes greater than 90% of the vasculature from the brain homogenate (Triguero et al., cited supra).

DEPR:

Capillary depletion studies following the procedures of Example 9 were performed with an OX-26-AZT conjugate using a pH-sensitive succinate linker. These studies employed a dual-labelled conjugate in which the AZT was .sup.14 C-labelled and the antibody carrier was .sup.3 H-labelled. The use of such a conjugate allowed independent monitoring of the disposition of both the antibody and AZT within the brain.

DEPR:

The linker was synthesized as follows. Succinic anhydride was used to acylate the AZT by reacting equimolar amounts of these two compounds for 3 hours at room temperature under argon in the presence of dimethylaminopyridine and sodium bisulfate in freshly distilled pyridine. The product was isolated by chromatography on a DEAE sephadex A50 column run with a triethylammonium bicarbonate buffer. The succinate derivative of AZT was activated at the carboxyl group as the NHS ester by reaction with equimolar amounts of N-hydroxysuccinimide and dicyclohexylcarbodiimide (DCC) in freshly distilled THF at 4.degree. C. for 2 hours. The product was purified by flash chromatography on silica gel. The resulting NHS-ester of AZT-succinate was used to acylate amine groups on OX-26, resulting in an AZT-OX-26 conjugate. A 15-fold molar excess of AZT-NHS ester was reacted with OX-26 in HEPES buffer overnight at 4.degree. C. The antibody-drug conjugate was isolated from free drug on a PD-10 column. The molar ratio of drug to antibody was 7:1. These studies employed a dual-labelled conjugate in which the AZT was .sup.14 C-labelled and the antibody carrier was .sup.3 H-labelled.

DEPR:

A soluble form of CD4, consisting of amino acids 1-368, was conjugated to OX-26 using a linkage that directed the attachment of the CD4 to the carbohydrate groups located in the Fc portion of the antibody. By directing the site of attachment in this way, the chance that the passenger molecules will interfere with antibody-antigen recombination is lessened. The linkage between the proteins was achieved by first introducing a sulfhydryl group onto CD4 using SATA (N-Succinimidyl S-acetylthioacetate), a commercially available compound. A hydrazid derivative of SPDP, another commercial cross-linking agent, was attached to OX-26 via carbohydrate groups on the antibody. Reaction of the two modified proteins gives rise to a disulfide-linked conjugate.

DEPR:

More specifically the linkage between the proteins was achieved by first introducing a sulfhydryl group onto CD4 using N-succinimidyl S-acetylthioacetate (SATA), a commercially available compound. A 4-fold molar excess of SATA was added to 5 mg of CD4 in 0.1M sodium phosphate buffer containing 3 mM EDTA (pH 7.5). This mixture was reacted at room temperature in the dark for 30 minutes. Unreacted starting materials were removed by passage over a PD-10 column. A hydrazid derivative of SPDP, another commercially available cross-linking agent, was attached to OX-26 via carbohydrate groups on the antibody. Ten milligrams of OX-26 in 2.0 ml of 0.1M sodium acetate, 0.15M sodium chloride (pH 5.0) was reacted with a 1000-fold molar excess of sodium periodate for 1 hour at

4.degree. C. in the dark. Unreacted starting materials were removed by passage over a PD-10 column. The oxidized antibody was reacted with a 30-fold molar excess of hydrazido-SPDP overnight at 4.degree. C. with stirring. Reaction of the two modified proteins gives rise to a disulfide-linked conjugate. One tenth volume of 0.5M hydroxylamine was added to the thioacetylated CD4 (CD4-DATA) and derivatized antibody was then added such that the ratio of CD4 to antibody was 7.5:1. This mixture was reacted at room temperature in the dark for 2 hours. Conjugate was purified by running the reaction mixture over a protein A column followed by a CD4 affinity column.

DEPR:

This model system was used to examine the efficacy of NGF delivered iv using an anti-TfR antibody. The treatment groups in this experiment consisted of OX-26-NGF iv, NGF iv, OX-26 iv, NGF icv and carrier buffer icv. The iv dosing regimen consisted of 30 .mu.g NGF, either as conjugate or free protein, or an amount of OX-26 equivalent to that in the conjugate, given daily for two weeks. Based on the percent of the injected dose of NGF that reaches the brain parenchyma as determined from the capillary depletion experiments, this dose of conjugate should result in the delivery of approximately 2.0 .mu.g of NGF. Taking into account the K.sub.D of the NGF receptor, this amount of NGF should be more than sufficient to stimulate the cholinergic neurons of the basal forebrain if the NGF is transported intact across the blood-brain barrier. The icv animals were treated via cannulas attached to Alzet osmotic minipumps.

DEPR:

Conjugation of superoxide dismutase (SOD) to OX-26 was performed following the strategy previously described for CD4 (see Example 13). SOD was modified with SATA to introduce a protected thiol group and OX-26 was modified through the carbohydrate group with PDP-hydrazide to yield a disulfied-linked conjugate. The yield of conjugate with this approach was approximately 10% (in terms of SOD).

DEPL:

In the two negative control animals that had complete lesions (1 NGF iv and 1 carrier buffer icv), approximately 40-50% of the neurons on the ipsilateral side of the lesion survived relative to those on the contralateral side of the lesion. In contrast, the percent of neurons surviving in two of the conjugate-treated animals was .about.80-90%; this is as good as, if not better than, what has been reported for NGF treatment icv. A third conjugate treated animal showed .about.60% neuron survival, which was less than that for the other two animals but still better than the negative controls.

DETL:

TABLE I _____ Results of Fimbria Fornix
Experiment #1 Result (% stained neurons, Treatment ipsilateral/contralateral)
NGF iv 41.5 .+- 8.3 carrier buffer icv
50.0 .+- 10.5 AK-26-NGF conjugate iv (#1) 60.8 .+- 19 AK-26-NGF conjugate iv
(#2) 96.5 .+- 32 AK-26-NGF conjugate iv (#3) 85.9 .+- 14